LESSON 17:
SOMACLONAL VARIATIONS

Introduction

What are genetic variations?

It is well known that genetic variations occur in undifferentiated cells, isolated protoplasts, calli, tissues and morphological traits of regenerated plants. The cause of variation is mostly attributed to changes in the chromosome number and structure. Cytological heterogeneity in cultures arises mainly due to such factors as:

1. The expression of chromosomal mosaicism or genetic disorders in cells of the initial explants
2. New irregularities brought about by culture conditions.

In tissue cultures, such types of changes were generally discarded since the main objective was to raise genetically stable cultures. Investigations have revealed that cell or tissue cultures undergo frequent genetic changes (polyploidy, aneuploidy, chromosomal breakage, deletion, translocation, gene amplifications and mutations) and these are also expressed at biochemical or molecular levels. Plant cell and tissue cultures, therefore, provide increased genetic variability relatively rapidly and without applying a sophisticated technology. The genetic variability in cultures expresses in the form of variant traits in regenerated plants which are then transmitted to the progeny through sexual (lettuce, tobacco) or vegetative (sugarcane, potato) propagation.

The genetic variability present among cultured cells, plants derived from such cells or progeny of such plants is called somaclonal variation. Another term, gametoclonal variation, has been proposed for the variability present among pollen derived cells/ plants, but the term is rarely used. Generally, the term somaclonal variation is used for genetic variability present among all kinds of cells/plants obtained from cells cultured in vitro. Plants regenerated from tissue and cell cultures show heritable variation for both qualitative and quantitative traits. Somaclonal variation has been described in sugarcane, potato, tomato etc.

Isolation of Somaclonal Variants

Mutants for several traits can be far more easily isolated from cell cultures than from whole plant populations. This is because a large number of cells, say 106-109, can be easily and effectively screened for mutant traits. Screening of as many plants would be very difficult, ordinarily impossible. Mutants can be effectively selected for disease resistance, improvement of nutritional quality, adaptation of plants to stress conditions, e.g., saline soils, low temperature, toxic metals (e.g., aluminium), resistance to herbicides and to increase the biosynthesis of plant products used for medicinal or industrial purposes.

The various approaches to the isolation of somaclonal variants can be grouped into two broad categories: (i) screening and (ii) cell selection.

1. Screening. It involves the observation of a large number of cells or regenerated plants for the detection of variant individuals. This approach is the only feasible technique for the isolation of mutants for yield and yield traits. In general, R1 progeny (progeny of regenerated, Ro, plants) are scored for the identification of variant plants, and their R2 progeny lines are evaluated for confirmation. Screening has been profitably and widely employed for the isolation of cell clones that produce higher quantities of certain biochemicals. Computer based automated cell sorting devices have also been used to sereen as many as 1000-2000 cells/second from which desirable variant cells were automatically separated.
2. Cell Selection. In the cell selection approach, a suitable selection pressure is applied which permits the preferential survival/growth of variant cells only. Some examples of cell selection are, selection of cells resistant to various toxins, herbicides, high salt concentration etc. When the selection pressure allows only the mutant cells to survive or divide, it is called positive selection. On the other hand, in the case of negative selection, the wild type cells divide normally and therefore are killed by a counter selection agent, e.g., 5 BUdR or arsenate. The mutant cells are unable to divide as a result of which they escape the counter selection agent. These cells are subsequently rescued by removal of the counter selection agent. Negative selection approach is utilized for the isolation of auxotrophic mutants.

The positive selection approach may be further subdivided into four categories: (i) direct selection, (ii) rescue method, (iii) stepwise selection and (iv) double selection.

In direct selection, the cells resistant to the selection pressure survive and divide to form colonies; the wild type cells are killed by the selection agent. This is the most cornmon selection method. It is used for the isolation of cells resistant to toxins (produced by pathogens), herbicides, elevated salt concentration, antibiotics, amino acid analogues etc.

In the rescue method, the wild type cells are killed by the selection agent, while the variant cells remain alive but, usually, do not divide due to the unfavourable environment. The selection agent is then removed to recover the variant cells. This approach has been used to recover low temperature and aluminium resistant variant cells.

The selection pressure, e.g., salt concentration, may be gradually increased from a relatively low level to the cytotoxic level. The resistant clones isolated at each stage are subjected to the higher selection pressure. Such a selection approach is called stepwise selection. It may often favour gene amplification (which is unstable) or mutations in the organelle DNA.

In some cases, it may be feasible to select for survival and/or growth on one hand and some other feature reflecting resistance to the selection pressure on the other; this is called double

selection. An example of double selection is provided by the selection for resistance to the antibiotic streptomycin, which inhibits chlorophyll development in cultured cells. The selection was based on cell survival and colony formation in the presence of streptomycin (one feature) as well as for the development of green colour in these colonies (second feature; only green colonies were selected). This approach has been used for the selection of cells resistant to the herbicide amitrole, 2, 4-D, tobacco mosaic virus (TMV) and aluminium.

Characterization of Variants

Somaclonal variants isolated through cell selection are often unstable. The frequency of stable variants may range from 8-62%, perhaps depending on the species and the selection agent. Many selected clones fail to exhibit their resistance during further screening or selection. Obviously these clones are susceptible and were misclassified as resistant (they are called escapes).

Several clones lose their resistance to the selection agent after a period of growth in the absence of selection pressure. Such clones are called unstable variants and may result from changes in gene expression and from gene amplification (increase in the number of copies of a gene per genome of the organism in comparison to that naturally present).

Some variant phenotypes are quite stable during the cell culture phase, but they disappear when plants are regenerated from the variant cultures, or when the regenerated plants reproduce sexually, in case they are expressed in the regenerated plants. Such changes are known as epigenetic changes and are attributed to stable changes in gene expression e.g., hormone habituation of cell cultures and, possibly, cold resistance in Nicotiana sylvestris.

The remaining variants which stably express the variant phenotypes during the cell culture as well the regenerated plant phases, and exhibit the transmission of these phenotypes through the sexual reproduction cycle are called mutants. Only this category of variants would find an application in crop improvement. These may represent true gene mutations or some other types of changes. Usually, expected mendelian ratios are obtained in the Rl progenies. But sometimes aberrant segregation ratios are encountered in Rl possibly due to the chimaeric nature of Ro plants, the involvement of some cytological anomalies like aneuploidy, deletions etc., gene dosage effects etc.

Molecular Basis of Somaclonal Variation

Somaclonal variation may arise due to any of the following events at molecular level: changes in chromosome number and/or structure, gene mutation, plasmagene mutation, alteration in gene expression, gene amplification, mitotic crossing over, transposable element activation, and rearrangements in cytoplasmic genes.

Most mutants isolated from cell cultures may involve single gene mutations, the mutant allele being either dominant or recessive. Gene amplification has been reported in some variants, usually recovered though stepwise selection of plant cells in vitro. Sometimes, deamplification may also occur in somac1onal variants, e.g., for rRNA genes. In addition, some previously silent mutator genes may become activated during tissue culture e.g., in maize, eleven out of the 301 somaclones showed Ac activity when they were test-crossed. Clearly, transposable elements can be activated during in vitro culture. The breakage and fusion of chromosomes, which occur during culture, may activate the Ac and/or other controlling elements.

Mitotic crossing-over could also account for some of the genetic variations detected in regenerated plants leading to the recovery of. homozygous recessive single gene mutations in Ro itself. Cytoplasmically inherited mutations have been recovered in several cases, e.g., T-toxin resistance, male-fertility and mt DNA restriction pattern changes in T -cms maize.

Most of the mutations recovered in tomato were identical with the already known spontaneous or induced mutations, but some mutations were entirely new (either new alleles of known genes or mutant alleles of hitherto unknown genes, e.g., jointless pedicel mutant in tomato which is highly valued for mechanical picking).

Somaclonal Variations and Induced Mutations

In general, mutagenic treatments are not applied to cell cultures for the recovery of somaclonal variants. But in those studies where mutagenic treatments were used, usually an increase in the frequency of somaclonal variants was observed. In some cases, mutagenesis was reportedly necessary for the recovery of the specific variant being isolated. However, mutagenesis should be avoided as far as possible in view of the undesirable features associated with such treatments.

Somaclonal variations are preferable to induced mutations for several reasons:

i. chimaerism is a major problem in induced mutations but not in somaclonal variations,

ii. induced mutations are often associated with undesirable features like sterility etc.,

iii. 'new' alleles and even 'new' mutations have been recovered through somaclonal mutations,

iv. the frequency of useful mutations is very high in the case of somaclonal variations,

v. a highly effective selection can be applied in vitro for several economically important characters which is virtually impossible in the case of mutation breeding,

vi. an astronomically large number of individuals can be effectively screened in vitro, etc. But, it may be emphasized that the somaclonal variation is applicable to only those species where whole plant can be regenerated from cultured cells, while mutation breeding can be applied to all the species, and that the former is dependent on quite sophisticated facilities for tissue culture and of greenhouse.

The frequency of useful somaclonal variations appears to be surprisingly high (Table.1). In sugarcane, 23% of the 235 somaclones were as resistant to Fiji disease as the control, while 5% were more resistant. Out of the 500 somaclones of potato, 1 % were more resistant to early blight (Alternaria solani) than the parent variety. In another study, 2.5% of the 800 potato somaclones were resistant to race 0 of late blight (Phytophthora

infestans), while 0.5% were resistant to races 1, 2, 3 and/or 4. A peculiar finding has been reported in apple where all of the 184 somaclones tested were more resistant to scab than the parent variety.

TABLE 1. The frequency of somaclones resistant to a specific disease; these somaclones were identified through screening, and there was no selection during the in vitro phase.

		Somaclones	Resistant	
Crop	Disease	screened	somaclones	Remarks
			(%)	
Sugarcane	Fiji disease	235	23	Comparable to control
			5	More resistant than
				Control
Potato	Early blight	500	1	More resistant than parent
	(Alternaria Solani)			Clone
	Late blight	800	2.5	Resistant to race 0
	(Phytophthora infestans)		0.5	Resistant to races 1,2,3,
				and/or 4
Apple	Scab	184	100	More resistant than parent
				variety.

Applications

Somaclonal variations have been isolated for a variety of traits. Some of the important ones are briefly described below.

Disease Resistant Variants

Somaclonal variants resistant to diseases can be isolated by screening a large number of plants regenerated through tissue culture of which some may show useful resistance. Alternatively, they may be isolated by selecting cells, protoplasts, calli, embryos or meristems for resistance to the concerned toxins by placing them on a medium containing a lethal concentration of the toxin. Calli, cells, embryos or plants which survive and grow on this medium are expected to be resistant to the toxin and, in many cases, to the pathogen as well. The toxins may be in the form of culture filtrate, a crude preparation or in the purified form.

Many pathogenic bacteria and fungi produce toxins that are toxic to plant cells. These toxins may be either specific or nonspecific.

Specific toxins exhibit a very high specificity for the host species and the host varieties which very closely parallels the susceptibility of the latter to the pathogen strain which produced the toxin, e.g., oat varieties and the toxin produced by Helminthosporium victoriae (victoria blight), maize varieties and the toxin produced by Helminthosporium maydis race T etc.

But the toxins produced by many pathogens are toxic to plant species and varieties of crops in general, without any reference to their susceptibility to the races of the pathogen which produced these toxins. Such toxins are called nonspecific toxins, e.g., toxins produced by many Alternaria sp., many pathogenic soil bacteria etc.

Plant cell cultures may be exposed to lethal concentrations of these toxins and resistant clones isolated. Plants regenerated from the toxin resistant clones would be resistant to the disease producing pathogen as well, provided the toxin is involved in disease development. An example of such an application is in the case of wildfire disease of tobacco (N. tabacum) produced by Pseudomonos tabaci. Tobacco cells resistant to methionine sulfoximine, which is similar to the toxin produced by the pathogen, were isolated. Plants regenerated from these clones were resistant to wildfire disease, although to a somewhat lesser degree.

Similarly, maize (Z mays) lines having the Texas male sterile cytoplasm are susceptible to Southern leaf blight caused by Helminthosporium maydis, which produces a toxin that binds to the mitochondria. Maize cells resistant to this toxin have been selected, and plants regenerated from them were resistant to leaf blight caused by H. maydis. These are two examples from a long list of successful reports on the isolation of disease resistant lines through selection of cells resistant to the toxin produced by pathogens. It may be expected that this approach may become increasingly valuable in view of the ease in selection of resistant clones and particularly in those cases where a satisfactory source of resistance is not found in the germplasm of host crop species.

It may be emphasized that this approach is successful only in the case of those pathogens which produce toxins which are involved in disease development. But many of the pathogens either do not produce a toxin or produce a toxin with an uncertain role in disease development. The cell selection approach can not be successfully applied in such cases. In the case of nonspecific toxins, the toxin resistant lines may be susceptible to the pathogen e.g., in case of bacterial wilt of tomato. In all such cases, somaclones may be obtained from unselected cell cultures, and the Rl progeny of these somaclones may be screened for resistance to the concerned pathogen. This approach was successful in the isolation of a bacterial wilt resistant tomato line, and of a Fiji disease resistant sugarcane line (from variety 'Pindar') which was subsequently released as a new variety (variety 'Ono').

Stress Resistant and Other Mutants

Plant cells resistant to 4-5 times the normally toxic salt (NaCl) concentration have been isolated. In many cases, the plants regenerated from them were also tolerant to saline conditions. For example, tobacco plants regenerated from high salt (0.88%) tolerant cell lines were also salt tolerant, and this feature was passed onto two successive sexual generations. But in some cases, salt tolerant cell lines become salt-dependent in that they require the presence of high salt levels for optimum growth, e.g., alfalfa and citrus cell lines. This approach may permit the development of crop varieties suitable for cultivation in saline soils.

Low temperature is another important environmental factor affecting survival and performance of crop plants. Cell lines resistant to chilling have been isolated in several cases, e.g., chillies, Nicotiana sylvestris etc. In some soils, an excess of

metal ions, e.g., aluminium, may adversely affect the performance of crop varieties. Varieties suitable for cultivation on such problem soils may be developed by selecting cell lines resistant to the concerned ion. The feasibility of this approach has been amply demonstrated by the selection of aluminium resistant cell lines of N. plumbaginifolia and regeneration of resistant plants from these lines.

Mutants for Agronomic Characters

In vitro regenerated plants often show variation in quantitative characters. Such variants have been described in a number of crops including tobacco, corn, barley, tomato, sorghum, wheat, carrot, Brassica, celery, sugarcane, potato etc. There is atleast one example of a commercial variety being developed using such variants. The alfalfa variety 'Sigma' was developed as a polycross among superior somaclones derived from certain commercial varieties. Sigma shows improved yield as well disease resistance.

Mutants for Efficient Nutrient Utilization

In vitro selection may be a useful method for developing phosphate ultraefficient crop plants. Isolation of somaclonal variant tomato cell lines which are able to grow normally in phosphate deficient condition due to a high secretion of acid phosphatase, which greatly increased the rate of phosphate uptake, has been reported.

Achievements

Over a dozen varieties have been developed through the exploitation of somaclonal variation. 'Ono' variety of sugarcane is a Fiji disease resistant somaclone of the susceptible cultivar 'Pindar'. It was identified by screening of plants regenerated from unselected calli. 'Ono' also shows yield advantage over 'Pindar' and has been cultivated to a limited extent in Fiji. A sweet potato cultivar 'Scarlet' was selected from shoot-tip culture-derived clones. 'Scarlet' is comparable to the parent cultivar in yield and disease resistance, but shows darker and more stable skin colour, which is a desirable quality trait. A geranium variety called 'Velvet Rose' is a somaclone of 'Rober's Lemon Rose'. The new variety has twice the chromosome number of the parent variety. An alfalfa variety called 'Sigma' is a polycross of selected somaclones.

In India, so far somaclonal variation is the only biotechnological approach to give a commercial variety. A somaclonal variant of Citronella java, a medicinal plant, has been released as 'Bio-13' for commercial cultivation by CIMAP (Central Institute for Medicinal and Aromatic Plants), Lucknow. Bio-13 yields 37% more oil and 39% more citronellol than the control varieties. A somaclonal variant of the B. juncea variety 'Varuna' has been released for commercial cultivation as 'Pusa Jai Kisan'. The new variety has bolder seeds and some yield advantage over the parent variety Varuna.

Advantages

1. Somaclonal variations occur in rather high frequencies which is a great advantage over conventional mutagenesis.
2. Some 'new' alleles or even 'new' mutations may be isolated which were not available in the germplasm or through mutagenesis, e.g., jointless pedicel mutant in tomato.
3. Use of somaclonal variation may reduce by two years the time required for the release of new variety as compared to mutation breeding. This is because somaclonal variations are usually free from undesirable features like sterility, while induced mutations are generally associated with such defects which necessitates one or two backcrosses with the parent variety.
4. A very effective selection can be practised at the cell level for several traits, e.g., disease resistance etc. This approach effectively selects few desirable cells from among millions with relatively small effort, time, cost and space requirements.
5. This is the only approach for the isolation of biochemical mutants, especially auxotrophic mutants, in plants.

Limitations

1. The technique is applicable only to those species of cell cultures which regenerate complete plants.
2. Selected cell lines often show reduced or no regeneration potential.
3. Many selected clones show undesirable features like reduced fertility, growth and even overall performance.

Conclusion

Somaclonal variation represents a useful source of introducing genetic variations that could be of value to plant breeders. Single gene mutation in the nuclear or organelle genome may give the best available variety in vitro that has a specific improved character. In this manner, somaclonal variation could be used to uncover new variants retaining all the favourable characters along with an additional useful trait, such as resistance to diseases or a herbicide. Various cell lines selected in vitro may then prove potentially applicable to agriculture and industry.

Questions

1. Define somaclonal variation. Briefly describe their isolation, characterization, molecular basis and applications.
2. What are the achievements, advantages and limitations of somaclonal variation.

Note